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Selection of various *Streptomyces* species by *Enchytraeus crypticus* (Oligochaeta)

V. Krištůfek[1,2], M. Hallmann[3], W. Westheide[3] and H. Schrempf[2]

[1] Institute of Soil Biology, Czech Academy of Sciences, České Budějovice, 37005, Czech Republic*
[2] Universität Osnabrück, FB Biologie/Chemie, Angewandte Genetik der Mikroorganismen, Barbarastraße 11, 49069 Osnabrück, Germany*
[3] Universität Osnabrück, FB Biologie/Chemie, Spezielle Zoologie, 49069 Osnabrück, Germany

Summary. Individuals of *Enchytraeus crypticus* (Oligachaeta) are attracted by 15–20% of *Streptomyces* species, mycelia-forming, gram-positive bacteria which had been isolated from the gut of earthworms as well as from soil. *E. crypticus* preferred living mycelia to autoclaved mycelia from the attractive strains. Comparative studies revealed that diffusable metabolites from *S. lividans* and *S. nogalater* were chosen, whereas those from *S. reticuli* were repulsive for *E. crypticus*. Streptomycetes can serve as sole nutrient source for *E. crypticus*, whose propagation rate correlated with the attractivity of the mycelia.

Key words: *Enchytraeus crypticus*, feeding preference, *Streptomyces* species

Introduction

The potworms Enchytraeidae are Oligochaeta which are highly abundant (up to $3 \times 10^5/m^{-2}$) in the upper layers of acid organic soil with a relatively high moisture content (O'Connor 1967; Petersen & Luxton 1982). Within moder humus woodland sites, the number of enchytraeids may comprise 5 to 10% of the total animal biomass. Enchytraeids mainly consume decaying organic material and therefore it is assumed that they play an important part in the turnover of nutrients in soil. A few studies demonstrated that enchytraeids digest plant material, i.e. leaves, potatoes, oats, or seaweed (Reynoldson 1939; Dózsa-Farkas 1976; Dózsa-Farkas 1978; O'Connor 1967; Dash & Cragg 1972; Dash et al. 1981), algae (Nekrasova et al. 1976; Shtina et al. 1981), yeast (Brockmeyer et al. 1990), and bacteria (Dougherty & Solberg 1960; Brockmeyer et al. 1990). Additional observations led to the conclusions that enchytraeids browse rather on microbially decomposed than on fresh leaves (Dózsa-Farkas 1976; Dózsa-Farkas 1978).

Four our studies, we chose *Enchytraeus crypticus* Westheide & Graefe 1992, a species which belongs to the family Enchytraeidae and is living in composts. Contrary to earthworms, *E. crypticus* is easy to cultivate in laboratory conditions, its generation time is short, and it can therefore be well observed during the tests (Westheide and Bethke-Beilfuss 1991).

Streptomycetes are Gram-positive bacteria which form substrate and aerial mycelia as well as spores. Depending on the composition of the soil, their number can amount to 10^6 to 10^8 per g of dry soil, and thus they may constitute 10 to 50% of the bacteria (Alexander 1977). Streptomycetes were shown to be very important for the degradation of organic

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* *Corresponding authors:* H. Schrempf, V. Krištůfek

material including polysaccharides, protein, lipids, and nucleic acids. Moreover, they are the main producers of antibiotics and antifungal compounds (Kutzner 1981).
This paper describes for the first time interactions between various *Streptomyces* species and Enchytraeidae represented by *E. crypticus*.

Materials and Methods

Cultivation of enchytraeids

E. crypticus individuals were propagated in the dark at 21 °C on Petri dishes containing 1.2% agar in tap water, pH 7.2, and 2.0% oat flakes. For food preference studies, the individuals were washed three times in sterile tap water.

Streptomyces species

About 51 various *Streptomyces* strains had been isolated from the gut content of different earthworms, i.e. *Aporrectodea rosea* (Savigny, 1826), *Lumbricus rubellus* Hoffmeister 1843 (Wessely 1905), *Octolasion lacteum* (Örley, 1881) and *Octolasion montanum* (Wessely 1905) as well as from soil meadows, South Bohemia, Czech Republic (Krištůfek, to be published). *Streptomyces lividans* 66 (Hopwood et al. 1985), *Streptomyces olivaceoviridis* (Beyer & Diekmann 1985; Blaak et al. 1993); *Streptomyces reticuli* (Schrempf 1983) and *Streptomyces nogalater* (Krištůfek et al. 1990 & 1993) had been described earlier.

Selection tests

In order to pretest which *Streptomyces* strains might be attractive for *E. crypticus*, five strains were patched on agar (1.2%) containing minimal medium (Schrempf 1983) supplemented with 2% flaked oats and incubated for up to five days at 30 °C. After this period, the substrate mycelia of the *Streptomyces* species has developed aerial mycelia and spores. 50 individuals of *E. crypticus* were then so arranged in the centre of the Petri dishes that their distance to each patch was nearly identical. The plates were kept at 21 °C, and the *E. crypticus* individuals surrounding each patch were counted after 2, 5 and 9 days, respectively, with the help of a binocular microscope. For detailed studies, mycelium of one individual *Streptomyces* species was placed on one plate, and then the *E. crypticus* individuals were put in the centre of the plate. The numbers surrounding the patch of the *Streptomyces* strain were counted daily.

Effect of diffusable metabolites

Sterile round cellophane paper was placed on the surface of a Petri dish containing minimal medium solidified with 1.2% agar and supplemented with 2% flaked oats. Then one *Streptomyces* species was streaked as patch. After five days of incubation, the cellophane paper was taken off, together with the mycelial colonies. Thus, diffusable metabolites produced by *Streptomyces* species were retained in the agar. 50 individuals of *E. crypticus* were placed on each plate and the numbers surrounding the part of the plate which contained the diffusable metabolites were counted. Comparisons of different plates were made, each of them possessing the metabolite(s) from another *Streptomyces* species.

Feeding tests with mycelia

Streptomyces species were cultivated individually in flasks containing minimal medium (Hopwood et al. 1985) supplemented with 2% flaked oats. After five days of cultivation, mycelia were harvested by low speed centrifugation (5,000 × g), washed four times with sterile water, and resuspended in 5 ml of sterile water. 2.5 ml were autoclaved, the other part was not. Each of the mycelia (weighing 0.1 g in wet state) was put on a separate Petri dish (∅ 9 cm) containing only 1.2% agar in tap water. Young adults of *E. crypticus* were placed on each plate and cultivated in the dark at a temperature of 21 °C. The *E. crypticus* individuals (juveniles and adults) were being counted over a period of four weeks. The mean fresh mass of *E. crypticus* was determined per plate after 3 and 4 weeks of cultivation. Having counted the number of individuals, the mean weight of one individual was calculated.

Statistical analysis

The analysis of variance was performed on *E. crypticus* population experimental data. The level of significance was chosen to be Alpha ≤ 0.01 (Student-Newman-Keuls test).

Results

Selection of **Streptomyces** *species by* **E. crypticus**

Of eight *Streptomyces* species originating from the gut of *Lumbricus rubellus*, two (*Streptomyces nogalater* and *Streptomyces* species 26) were attractive for *E. crypticus*, as well as one strain (*Streptomyces* species 16) out of 13 isolates from the gut of *O. montanum*. One strain (*Streptomyces* species 19) out of 10 isolates from the gut of *O. lacteum*, and one strain (*Streptomyces* species 29) out of 9 isolates from the gut of *A. rosea* were selected by *E. crypticus*, too; the same applies to two out of 11 strains taken from the same soil sample in which the earthworms has been isolated (Krištůfek et al. 1993; Krištůfek, to be published). In order to learn if *Streptomyces* species are also attractive, we studied *S. lividans*, *S. olivaceoviridis*, and *S. reticuli* originally isolated from soil in different countries and selected for our culture collection.

The results of the studies (Table 1) revealed that *S. lividans* was the most attractive strain for *E. crypticus*; *S. nogalater* ranked second. *S.* species 26 and *S. olivaceoviridis* were only selected by few *E. crypticus* individuals. *S. reticuli* was avoided by *E. crypticus* (Fig. 1). Further studies concentrated on the two attractive strains *S. lividans* and *S. nogalater* and

Table 1. Selection of various *Streptomyces* species by *Enchytraeus crypticus*

Streptomyces species and their origins	Attraction of *E. crypticus*[a]
Streptomyces **isolates from guts** (worms)	
S. nogalater (Lumbricus rubellus)	+ +
S. species 26 *(Lumbricus rubellus)*	+/−
S. species 16 *(Octolasion montanum)*	+
S. species 19 *(Octolasion lacteum)*	+
S. species 29 *(Aporrectodea rosea)*	+
Streptomyces **strains from soil**	
S. species 1	+/−
S. species 10	+
Strains from culture collections	
S. lividans	+ + +
S. olivaceoviridis	+
S. reticuli	−

[a] After a preselection test (see Materials and Methods), one *Streptomyces* species was plated as patch on an agar plate containing oatmeal medium. The numbers of *E. crypticus* individuals were counted over a period of 9 days. The attractivity was increasing from zero (−) to +/−, +, + +, and + + +

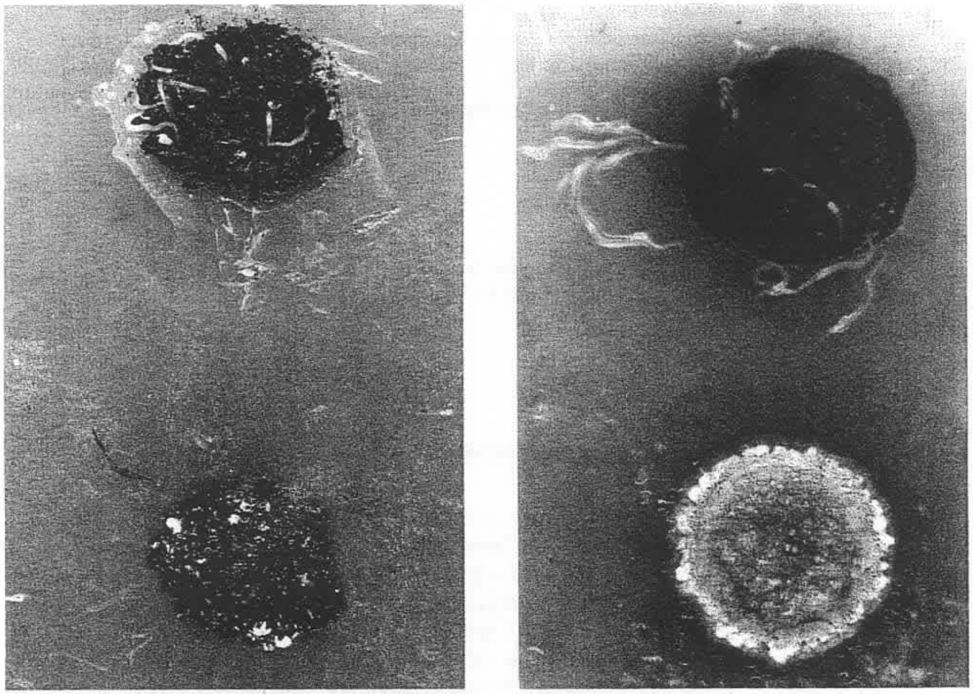

Fig. 1. Right: Colonies of *Streptomyces* species are selected (top; *S. nogalater*) or avoided (bottom; *S. reticuli*) by *E. crypticus*. Left: *E. crypticus* prefers living (top) to autoclaved mycelia (bottom) from *S. nogalater*

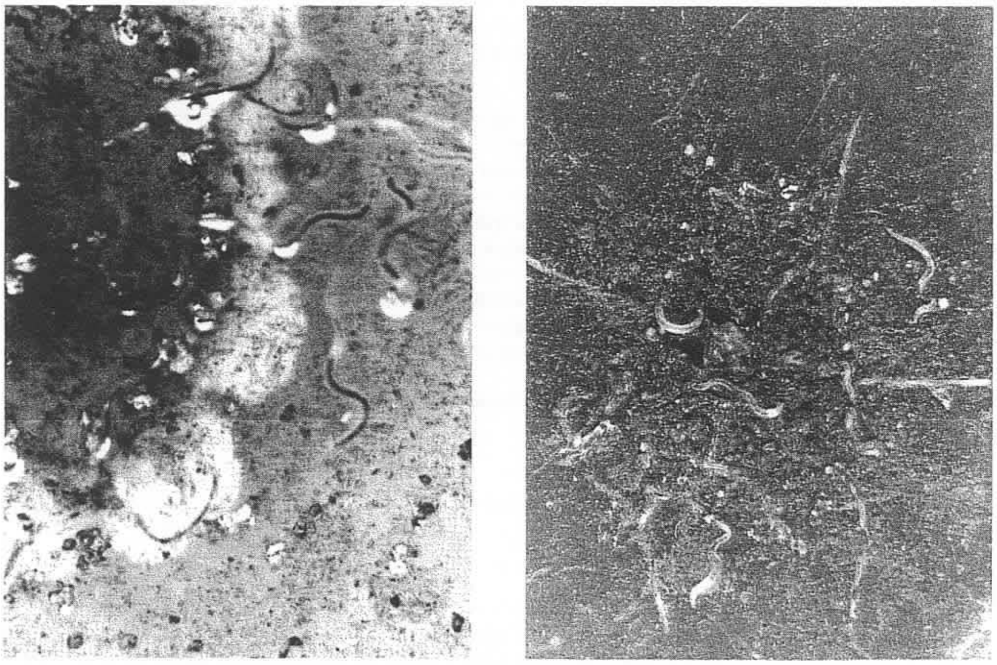

Fig. 2. *E. crypticus* ingests either spores (left) or mycelia (right) from an attractive *Streptomyces* strain

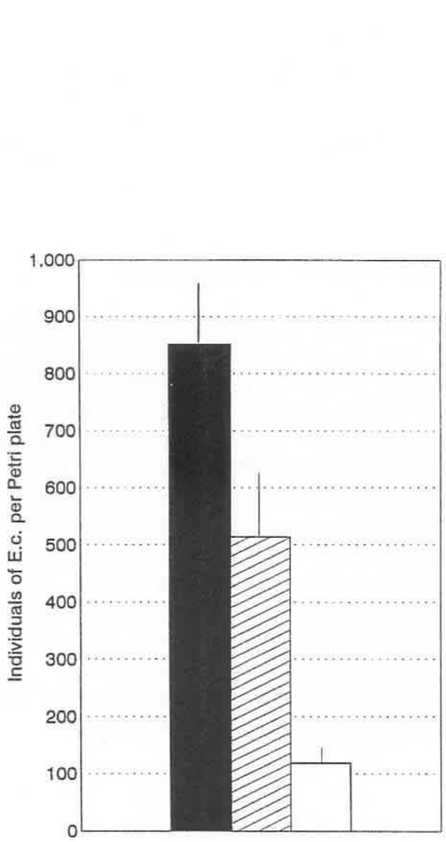

Fig. 3. Effect of *Streptomyces* mycelia on the development of *E. crypticus*. 10 young *E. crypticus* adults were placed on a Petri dish containing one patch (0.1 g) of mycelium. Over a period of 4 weeks, 0.1 g mycelium was newly added as food every 7 days. For each of the strains, *S. lividans* ■, *S. nogalater* ▨, and *S. reticuli* □, the experiments were performed 5 times (mean ±s.e.)

the non-attractive *S. reticuli. E. crypticus* was exposed to the diffusable metabolites of each of the *Streptomyces* species, as described under Materials and Methods. The highest and second highest numbers of *E. crypticus* individuals surrounded the metabolites from *S. lividans* and *S. nogalater*, respectively. The *S. reticuli* metabolites were avoided. Spores and mycelia were indigested from the attractive strain (Fig. 2).

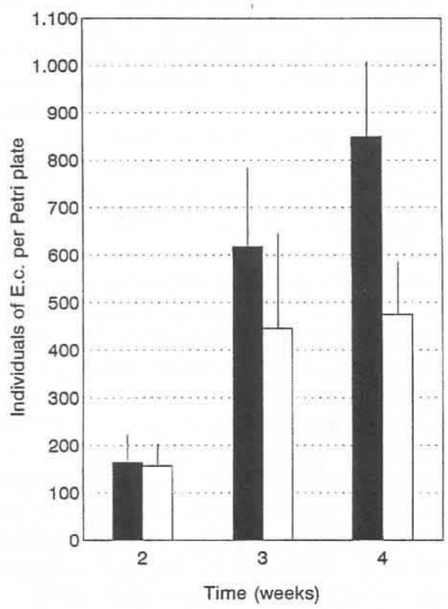

Fig. 4. Population dynamics of *E. crypticus*. 10 young *E. crypticus* adults were placed on a plate containing living (■) or autoclaved (□) mycelia from *S. nogalater*. As described in Fig. 3, 0.1 mg of mycelium was placed each week in the plate. The number of *E. crypticus* individuals was counted on five plates which had been arranged parallely and the mean ±s.e. was calculated

Streptomyces *mycelia as nutrient source for* **E. crypticus**

E. crypticus was offered mycelia from the two attractive *(S. lividans, S. nogalater)* and one non-attractive *(S. reticuli)* species as sole nutrient source. The number of *E. crypticus* individuals was highest (852 ± 112) and second highest (514 ± 118) in *S. lividans* and *S. nogalater*, respectively. Contrary to this, only 119 ± 16 *E. crypticus* individuals were counted with *S. reticuli* mycelia as diet (Fig. 3). An analysis of the counts showed (Fig. 4) that the number of *E. crypticus* individuals subsisting on living mycelia of *S. nogalater* increased after four weeks of incubation about 2-fold (849 ± 172), compared to those fed with its autoclaved mycelia. In the presence of autoclaved mycelia, the population remained constant between the 3rd and the 4th week of cultivation, whereas it grew by about 40% during this time period when living mycelia served as diet. The mean fresh mass of one worm was found to be identical (1.608 ± 0.289 mg) after three weeks of cultivation with both types of mycelia. After *E. crypticus* had been fed with living mycelia for four weeks, the mean mass of one individual dropped to 0.658 ± 0.125 mg. In contrast, in the presence of autoclaved mycelia, the number of individuals was lower and the average mass of an *E. crypticus* individual amounted to 1.222 ± 0.088 mg.

Discussion

Our studies revealed for the first time that *E. crypticus* is attracted by various *Streptomyces* species. Approximately 15% and 20% of the *Streptomyces* species isolated from earthworms and soil, respectively, were chosen by *E. crypticus*. Two out of three *Streptomyces* strains which had randomly been selected from culture collection were also attractive, whereas one was repulsive. When living and dead mycelia from attractive strains were offered to *E. crypticus* as the only food on the same plate, the worms selected only the living mycelia as nutrients (Fig. 1). In addition, we found that diffusable metabolites which are secreted by *Streptomyces* species are preferred by *E. crypticus*. These investigations show that metabolites adhering to mycelia and (or) diffusing to the media attract *E. crypticus*. Its enumeration was most positively affected by the living mycelia, compared to the autoclaved one. These data suggest that attractive metabolites had been destroyed during the autoclaving process. The propagation rate increased with the degree of attractivity of the *Streptomyces* species (*S. lividans* > *S. nogalater* > *S. reticuli*, see Table 1 and Fig. 3). It is possible that compounds within the living mycelia support the reproduction of *E. crypticus*. This pilot study demonstrated that *E. crypticus* is particular as to food quality.

Diets (quantity and quality) have been considered to be one of the most important ecological factors in determining population dynamics of invertebrates. Earlier studies (Dash et al. 1981) had indicated that enchytraeids are capable of digesting proteins as well as di- and some polysaccharides through the action of the enzymes within their guts. Enchytraeidae were found to possess enzymes hydrolysing starch, laminarin, cellulose, proteins (Nielsen 1962; Nielsen 1963; Urbášek & Chalupský, Jr. 1991), xylan and lichenan (Urbášek & Chalupský, Jr. 1991). It was also shown that starch, xylan and laminarin were degraded most efficiently (Urbášek & Chalupský, Jr. 1991); in addition, the enzymatic activities varied among different enchytraeid species (Dash et al. 1981; Urbášek & Chalupský, Jr. 1991). However, more complex plant substances are supposed to become accessible to enchytraeids through microbiological action (Dash et al. 1981; Diddan 1993). Consumption and indigestion of certain fungi, i.e. *Ceuthospora* species, *Cladosporium* species, *Penicillium* species, *Paecilomyces* species (Dash & Cragg 1972; Dash et al. 1980) and of bacteria (Dougherty and Solberg 1960) including *Bacillus cereus* var. *mycoides* (Brockmeyer et al. 1990) was reported. Apart from serving as nutrient, the microorganisms in the gut may provide their enzymatic repertoire for the worms, so that these are able to digest complex organic material more rapidly. In contrast to fungi, *Streptomyces* species are more difficult to identify. This explains why a possible role of streptomycetes in the gut of worms has not yet been elucidated.

Streptomycetes efficiently degrade chitin and other polysaccharides, such as cellulose, lignocellulose, starch, and xylans, and thus play an important part in the decomposition of organic material. It will be of special interest to test whether streptomycetes which are taken up by enchytraeids supply their enzymatic repertoire to enhance the efficiency of the degradation within the guts of potworms.

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